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The *cdc25* homologue *twine* is required for only some aspects of the entry into meiosis in *Drosophila*

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SUMMARY

The *twine*^{HB5} mutation prevents spindle formation during the entry into meiosis in *Drosophila* males, but chromosome condensation and nuclear envelope breakdown both still occur. This suggests the possibility that this particular *cdc25* homologue is required to activate a p34^{cdc2} kinase required for only some of the events of this G_2-M transition. In contrast, meiotic spindles do form in *twine*^{HB5} females, although these appear abnormal. However, the female meiotic divisions do not arrest at metaphase I as in wild type, but continue repeatedly, leading to gross non-disjunction. Small chromatin masses, corresponding in size to the fourth chromosomes, often segregate properly to the spindle poles. These can persist into the embryos derived from *twine*^{HB5} females, where they appear to participate in mitotic divisions on thin spindles. In addition, these embryos contain a small number of large chromatin masses that are not associated with spindles.

Key words: *Drosophila*, meiosis, *cdc25*, *twine*, mitosis

INTRODUCTION

cdc25 was first identified in fission yeast as a positive regulator of the 34 kDa mitotic kinase encoded by *cdc2* (Russell and Nurse, 1986), a function that is opposed by the negative regulator *wee1* (Russell and Nurse, 1987a; reviewed by Nurse, 1990). These proteins regulate the tyrosine 15 phosphorylation state, and thereby the activity, of p34^{cdc2}, thus controlling the G_2-M transition. *wee1* encodes a protein kinase (Russell and Nurse, 1987b), which can phosphorylate tyrosine in vitro (Featherstone and Russell, 1991; Parker et al., 1991). Two homologues of *cdc25* have been identified in *Drosophila* as the genes *string* and *twine* (Edgar and O'Farrell, 1989; Jimenez et al., 1990), both of which have been expressed in bacteria and shown to have tyrosine phosphatase activity and the capability to activate p34^{cdc2} in vitro (Kumagai and Dunphy, 1991; Gautier et al., 1991; L. Alphey and P. Clarke, unpublished). Moreover, both homologues are functional in fission yeast and will rescue a temperature-sensitive *cdc25* fission yeast mutant. This formed the basis for the original isolation of *twine* (Jimenez et al., 1990), although the sequence conservation between all *cdc25* genes enabled Courtot et al. (1992) also to clone the *twine* gene using a PCR approach.

Embryos homozygous for mutation in the *cdc25* homologue *string* can complete the first 13 mitotic cycles that take place in the syncytial embryo utilising maternally provided *string* gene product, but fail to undertake the fourteenth round of mitosis that normally occurs following cellularisation (Edgar and O'Farrell, 1989). Unlike the earlier syncytial division cycles in which mitosis is synchronous, cycle 14 has an extended G_2 period and mitoses occur in a series of spatially and temporally regulated domains (Foe, 1989). *string* transcription precedes these divisions by 25-35 minutes (Edgar and O'Farrell, 1989). If *string* is ectopically expressed in embryos under the control of the heat shock promoter, it will induce entry into mitosis throughout the embryo (Edgar and O'Farrell, 1990). Thus *string* seems to behave as the primary regulator of the G_2-M transition in the newly cellularised embryo. *string* expression is also seen in dividing tissues in larval development (Alphey et al., 1992). These expression patterns contrast with the distribution of *twine* transcripts, which, although present in the syncytial embryo as part of the maternal contribution, are otherwise not seen in somatic tissues throughout development (Alphey et al., 1992). *string* and *twine* expression show overlapping patterns during oogenesis, but are expressed in distinct regions of the testes (Alphey et al., 1992; Courtot et al., 1992). *twine* is expressed in the growing stage of primary spermatocytes in a manner that suggests a role in regulating the entry into meiosis, and analysis of a *twine* mutation has demonstrated a requirement for the gene not only in male, but also in female meiosis (Alphey et al., 1992; Courtot et al., 1992). In this paper we show that *twine* is not required for all aspects of the entry into male meiosis, and mutation in *twine* leads to a variety of abnormal meiotic spindles and unusual chromosome segregation in female meiosis.

MATERIALS AND METHODS

Preparation and staining of oocytes

This was carried out using an adaptation of the method of Theurkauf and Hawley (1992). *Drosophila melanogaster* were anaesthetised with CO_2 and transfered to a homogeniser. They were washed twice with Robb's medium (55 mM Na acetate, 40 mM K acetate, 100 mM sucrose, 10 mM glucose, 1.2 mM $MgCl_2$, 1 mM $CaCl_2$, 100 mM HEPES, pH 7.4) to remove the freshly laid eggs. Then 2-3 ml of Robbs medium was added and the flies were gently crushed using a very loose fitting pestle. They were passed through a loose mesh and the filtrate collected in a Petri dish. They were washed with approximately 5 ml of Robb's medium and the oocytes allowed to settle through the mesh by gravity. The carcasses were returned to the homogeniser and crushed again. They were filtered and washed as before and the filtrates were pooled. Stage 13-14 egg chambers were collected by selectively pipetting them into a 10 ml sterilin tube, allowing them to settle then removing the supernatant. The oocytes were transered to a microfuge tube for fixation. As much of the Robb's medium was removed as possible, and 1 ml of fixative (100 mM K cacodylate, pH 7.2, 100 mM sucrose, 40 mM K acetate, 10 mM Na acetate, 10 mM EGTA, 8% formaldehyde) was added. The oocytes were mixed in fixative on a blood mixer for 10 minutes. Egg chambers were allowed to settle, the fixative was removed and the oocytes were rinsed twice with PBS. Chorions and vitelline membranes were removed by rolling the egg chambers between the frosted surface of a glass slide and a 22 mm × 50 mm coverslip. The oocytes were rinsed into an embryo dish with PBS. They were permeabilised and blocked in PBS containing 1% Triton X-100 and 10% FCS for 1-2 hours at room temperature, rinsed in PBS containing 0.1% Triton (PBST) and then stained with antibodies as described for embryos (Glover and Gonzalez, 1993).

Preparation and staining of testes

Testes were prepared for immunostaining by the methanol/acetone protocol described by Ashburner (1989). Indirect immunofluorescence was carried out following the protocol of Gonzalez and Glover (1993).

Acetic acid testes squash preparations

Testes were disected as for immunostaining. They were then cut and squashed in 45% acetic acid as in the protocol of Gonzalez and Glover (1993). The testes were examined immediately by phase contrast, using a Nikon Microphot FX microscope and ×40 plan phase objective.

Preparation and staining of embryos

Embryos were collected over one hour intervals. They were fixed and stained as described by Gonzalez and Glover (1993), except that the vitelline membranes were removed manually rather than with methanol. Embryos were mounted in 85% glycerol containing 2.5% *n*-propylgallate and examined using a Bio-Rad MRC600 laser scanning confocal microscope.

Antibodies used

The anti-tubulin monoclonal antibody supernatant YL1/2 was obtained from Sera Lab and was used at a dilution of 1:10. The anti-lamin monoclonal T47 and the anti-tubulin monoclonal Bx69 were the generous gift of Harold Saumweber. Tissue culture supernatants of the hybridoma lines were used undiluted. FITC-conjugated anti-rat and -mouse secondary antibodies were obtained from Jackson Immunochemicals.

RESULTS

Meiosis is initiated but not completed in *twine* males

The process of spermatogenesis begins with four rounds of mitotic division in the germinal proliferation centre at the apical tip of the tubular testis. This produces a cyst of sixteen cells, which remain linked by cytoplasmic bridges or ring canals. These cells grow over a period of about 90 hours and then undergo the two meiotic divisions to produce a cyst of 64 cells still linked by ring canals. This is known as the onion stage. Each cell within the cyst contains two spherical structures, the nucleus and the Nebenkern, a mitochondrial aggregate. These postmeiotic onion-stage cells then go through the process of elongation to produce mature sperm. Older cysts are displaced down the testis by the production and growth of more cysts. Hence all the stages of spermatogenesis are represented in each testis as a temporal progression with young cysts towards the tip, meiotic cysts further down and maturing sperm towards the base. We have reported previously the transcription patterns of the two *Drosophila* cdc25 homologues in the testis. The *string* RNA was only detectable in the cells at the apical tip of the testis. The *twine* RNA is absent from the apical cells but is expressed during the growing stage; the RNA is degraded sometime around the onset of meiosis. This suggested that *string* is required for the early mitotic divisions and that *twine* is required for the initiation of the meiosis. This latter hypothesis was borne out by our observation that development was normal up to the end of the growing stages but that the cysts did not go through meiosis (Alphey et al., 1992). We wished to determine whether any meiotic events were initiated, however, and so treated testes with 45% acetic acid, which destroys most of the cellular morphology except for condensed chromosomes and sperm heads. These show up as phase-dark objects. As we had expected *twine* to be activating $p34^{cdc2}$ kinase to promote entry into meiosis we were surprised to find that chromosome condensation does occur in the $twine^{HB5}$ mutant. Moreover this finding is in contrast to that reported by Courtot et al. (1992) who reported that there was no chromosome condensation in this mutant. As we reported previously we were unable to observe any meiotic division in the *twine* mutant. Fig. 1A shows an acetic acid squashed preparation of a wild-type testis, the condensed chromosomes (arrowheads) have congressed to the metaphase plate and the cyst is in meiotic metaphase I. The cyst shown in Fig. 1B is from a similar preparation of a $twine^{HB5}$ testis, condensed chromosomes are clearly visible (arrowheads) but we could never detect congression of the separate chromosomes to the metaphase plate. However the 16-cell onion-stage cysts do undergo some further differention. The acetic acid preparations revealed disordered arrays of sperm heads on partially elongated sperm in $twine^{HB5}$ testes (Fig. 1D). This is in contrast to the wild type in which the sperm heads are tightly bundled (Fig. 1C). All of these mutant phenotypes were also observed in $twine^{HB5}$/*Df(2L)RN2*, indicating that mutant allele behaves as a null.

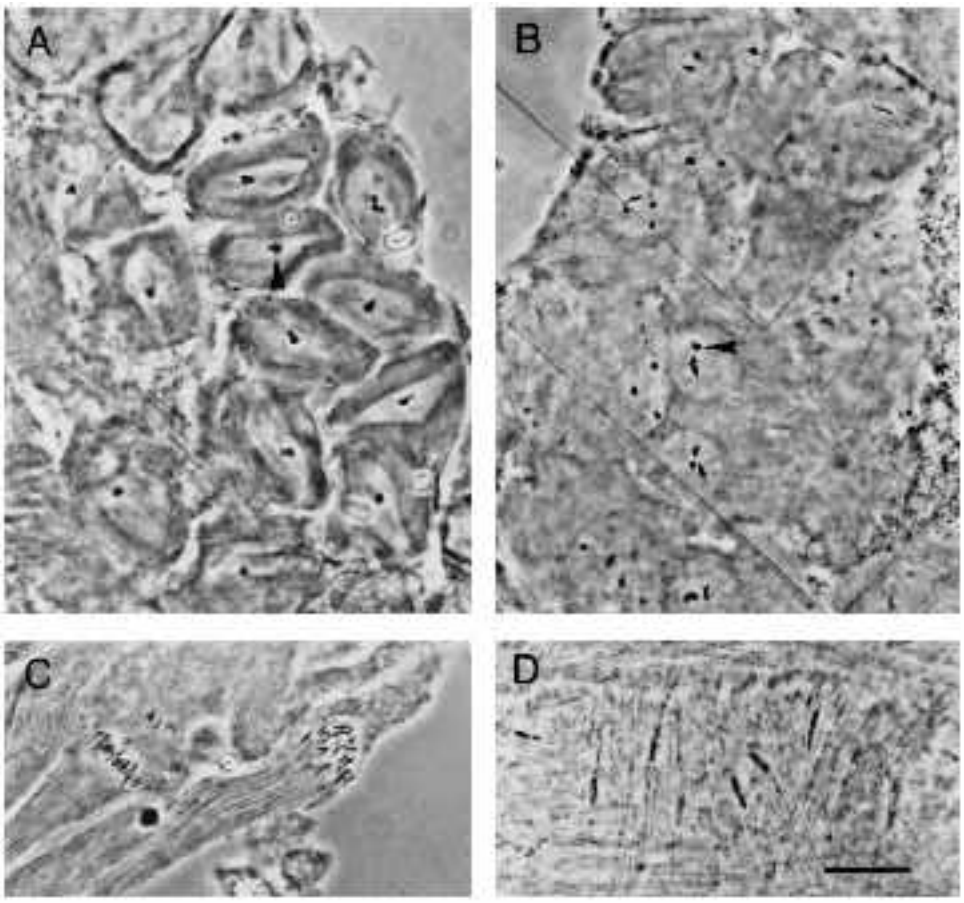

Fig. 1. Chromatin condensation in spermatogenesis in wild-type and *twine*^{HB5} testes. Preparations were squashed in acetic acid as descrided in Materials and Methods. (A) Condensed chromosomes (arrowhead) at metaphase I from a wild-type testis. (B) Condensed chromosomes (arrowhead), which do not congress to a metaphase plate from a *twine*^{HB5} testis. (C and D) Normal and disordered spermheads from wild-type and *twine*^{HB5} testes, respectively. That these condensed bodies indeed correspond to chromatin was confirmed using Hoechst (data not shown). Bar, 25 μm.

Nuclear envelope breakdown occurs in the absence of spindle formation in *twine* males

In order to characterise further the exact stage at which meiosis in *twine*^{HB5} males fails we carried out indirect immunofluorescence to study the behavior of nuclear lamins and microtubules. The condensation of DNA as if to enter meiosis I in *twine*^{HB5} males was confirmed using propidium iodide staining. The results of staining *twine*^{HB5} testes with a monoclonal antibody to lamins is shown in Fig. 2. Fig. 2A shows staining of the DNA in two adjacent 16-cell cysts; the cyst on the left has DNA that is only beginning to condense, the cyst on the right is at a sightly later stage and has more extensively condensed DNA. Fig. 2B shows the lamin staining of these two cysts. The cells in the cyst on the left have intact nuclear lamina (arrows) whereas those on the right have no lamin staining (arrows) indicating that the nuclear lamina is broken down as chromosomes condense. Wild-type cysts are shown in Fig. 2C-F. The premeiotic cells with decondensed DNA (C) have a nuclear lamina that is intact and indistinguishable from the *twine* cysts (D). The nuclear envelopes in the cyst at prophase II have broken down (E and F). In the wild type the lamin staining remains in a punctate pattern in the cells whereas in the *twine* cysts the staining completely disappears (compare Fig. 2B and F).

The phosphorylation of a large number of cellular proteins is a characteristic feature of entry into mitosis and meiosis. The monoclonal antibody MPM2 recognises an epitope that is phosphorylated in mitosis and meiosis on a number of antigens. Fig. 3A shows the DNA staining of a meiotic anaphase I cyst from a wild-type testis. The MPM2 antibody recognises the meiotic spindles within this cyst. The condensed chromosomes in a *twine*^{HB5} 16-cell cyst are shown in C. The MPM2 staining of this cyst (D) shows no staining of the meiotic spindle. This indicated that there is either no spindle formed in this mutant or that there is no phosphorylation of the MPM2 epitope in *twine*^{HB5} or both. We confirmed that there is no spindle formation using antibodies to tubulin. Fig. 4 shows wild-type and *twine*^{HB5} cysts stained with anti-tubulin antibodies. Fig. 4A and B show a wild-type cyst at anaphase I with meiotic spindles. Fig. 4C and D show a *twine*^{HB5} cyst in which chromosome condensation has occured but no spindle has formed.

twine oocytes fail to arrest in meiosis I and undertake aberrant divisions

Oogenesis in *Drosophila* begins with four mitotic divisions of a precursor cell in the germarium of the ovary to produce a cyst of 16 cells interconnected by cytoplasmic bridges. As the egg chamber matures one cell becomes the oocyte while the other cells develop into highly polyploid nurse cells. The stages of oogenesis are described in detail by King (1970). The nurse cells are responsible for supplying all the mRNAs and proteins that are required for oogenesis and early embryonic development and translocating them into the oocyte. mRNA for both *string* and

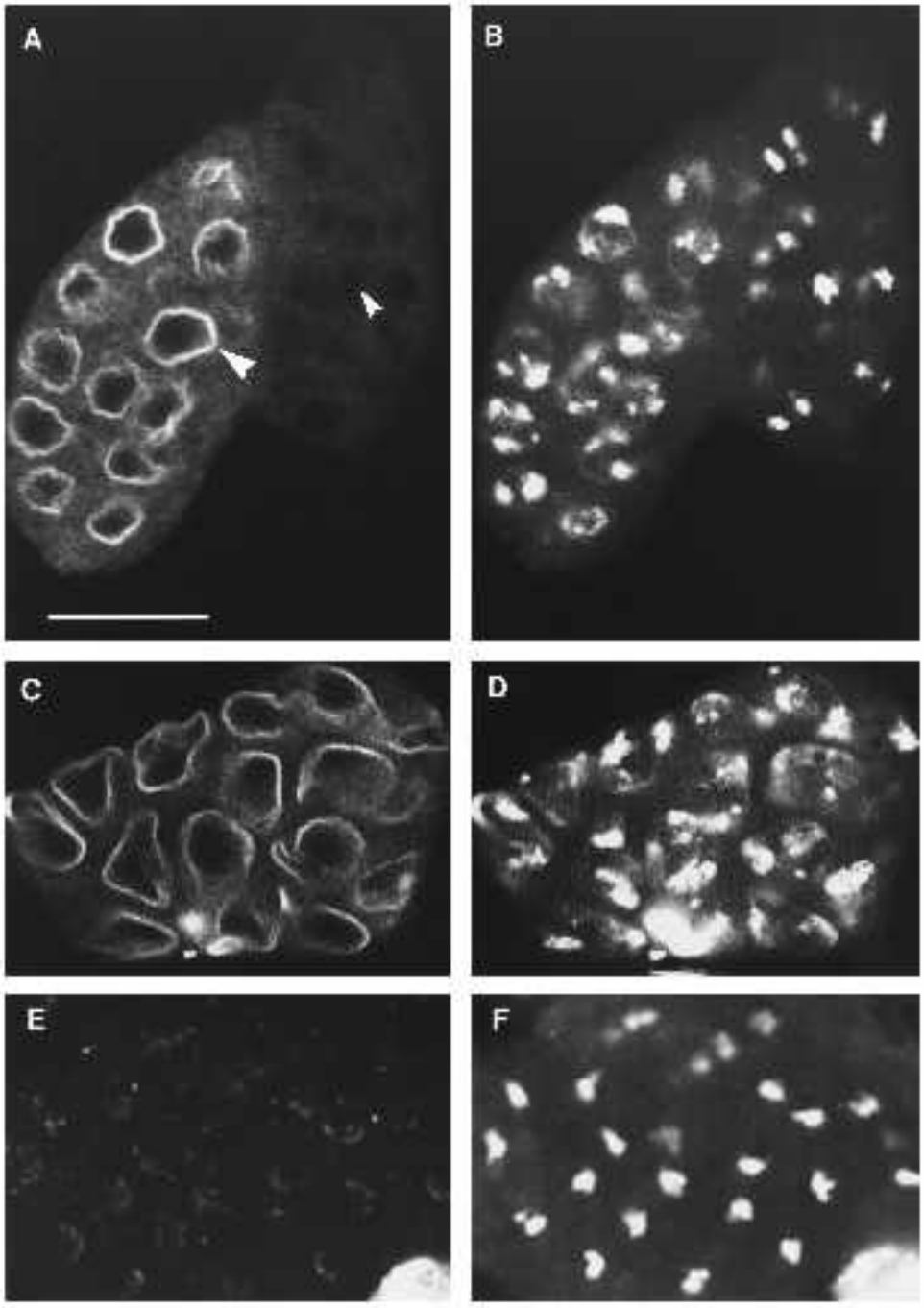

Fig. 2. Nuclear envelope breakdown occurs in *twine*^{HB5} testes. (A and B) A pair of cysts stained with propidium iodide and anti-lamin, respectively, from a *twine*^{HB5} testis. The large arrowhead in (A) points to an intact nuclear envelope from the premeiotic cyst. The small arrowhead indicates the absence of a nuclear envelope in the later stage cyst in which the chromatin is more condensed. (C-F) Wild-type preparations. (C and D) A premeiotic cyst, showing decondensed DNA and intact nuclear laminae. (E and F) Cells in prophase II, showing that the nuclear laminae have broken down. Bar, 25 μm.

twine is expressed in the nurse cells from stage 10; the messages are translocated into the oocyte by stage 13 (Alphey et al., 1992; Courtot et al., 1992). Prophase of meiosis is initiated in the presumptive oocyte nucleus in the germarium and continues during oogenesis. The early stages of oogenesis in *twine* mutants were found to be normal. Fig. 5A shows a stage 7 egg chamber from a *twine*^{HB5} female; the polyploid nurse cell nuclei (arrow) and the oocyte nucleus (arrow) can be clearly seen. The meiotic spindle is not formed until stage 13 of oogenesis. It forms as a short bipolar structure, which then lengthens before metaphase; an example of a metaphase spindle is shown in Fig. 5B (Therkauf and Hawley, 1992; Hatsumi and Endow, 1992). In mature stage 14 oocytes meiosis is arrested in metaphase I, with the exchange chromosomes in a bundle at the metaphase plate and the non-exchange fourth chromosomes displaced from the plate towards the poles (Fig. 5, inset in A). The meiotic divisions are completed only after activation of the oocyte on entry into the oviduct.

We have examined the behavior of the chromosomes and spindles in the mutant *twine*^{HB5} using indirect immunofluorescence. Defects were not seen until stage 14 of oogenesis in *twine* mutants when, instead of arresting at metaphase I, meiosis continues in a abnormal fashion. Abnormalities include spindles in which one pole was focused while the other was splayed, such spindles were

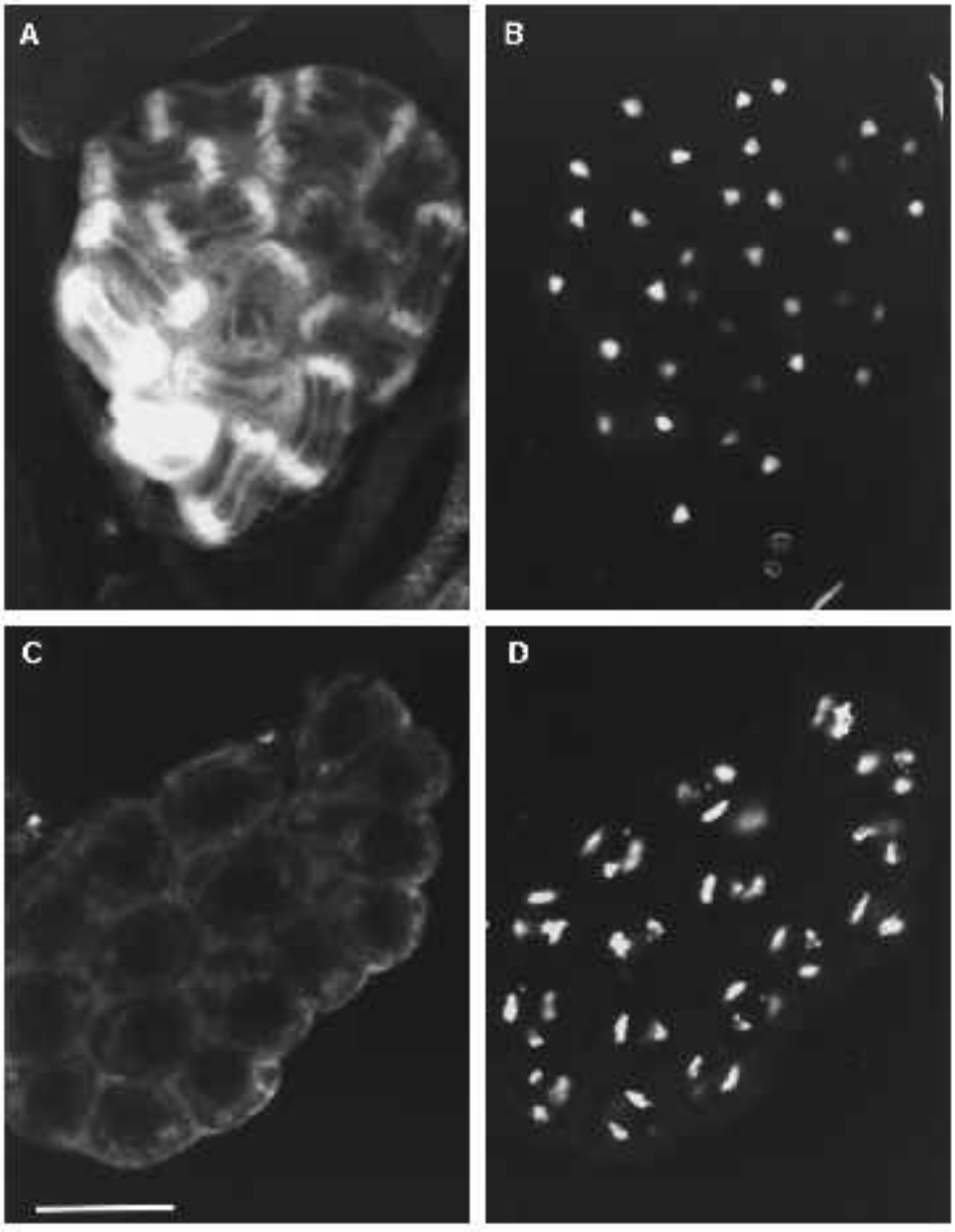

Fig. 3. MPM2 staining of wild-type and *twine*^{HB5} testes. (A and B) A wild-type cyst in meiotic anaphase I stained with the monoclonal antibody MPM2 and propidium iodide, respectively. MPM2 stains phosphorylated epitopes on the meiotic spindle. (C and D) Comparable staining in *twine*^{HB5} testes. In these cysts the chromosomes have condensed (D) but no spindle staining is visible (C). Bar, 25 μm.

often kinked as in Fig. 5C and D. Structures in which one pole is shared between two spindles were seen (Fig. 5F-I). The distribution of DNA varied between oocytes. Metaphase I-like figures were seen in which there was only one chromatin mass on a single spindle (C). Metaphase II-like structures with two spindles and two chromatin aggregates (D) were also found. Other oocytes contained four clumps of chromatin (E and F-I). These were normally found associated with spindle structures but occasionally were found displaced from the spindles as in Fig. 5E. Interestingly the fourth chromosomes appeared to sometimes escape from the mass of exchange chromosomes, much as they do in wild type (Fig. 5C and D, arrowed).

Aberrant mitoses in embryos derived from *twine* mothers

We have previously reported that approximately 50% of the embryos laid by *twine*^{HB5} homozygous females had up to five large nuclei, which undergo degradation after a few hours. We also observe this phenotype in *twine*^{HB5}/*RN2*-derived embryos. In an attempt to relate the aberrant meiotic phenotype found in oogenesis to subsequent embryonic development we examined the behavior of microtubules in *twine*^{HB5} embryos by immunostaining. We were able to detect some mitotic spindle-like structures in a proportion of the embryos. A few of these spindles were fairly normal in size and were associated with a 4N or greater complement of chromosomes. However the majority were very thin although of normal length, and were associated with tiny pieces of chromatin of a size consistent with the fourth chromosomes. An example of a normal-sized spindle sharing a pole with a 'thin' spindle is shown in Fig. 6. In general, embryos contained several of these thin spindles with associated small chromosomes, which were often localised

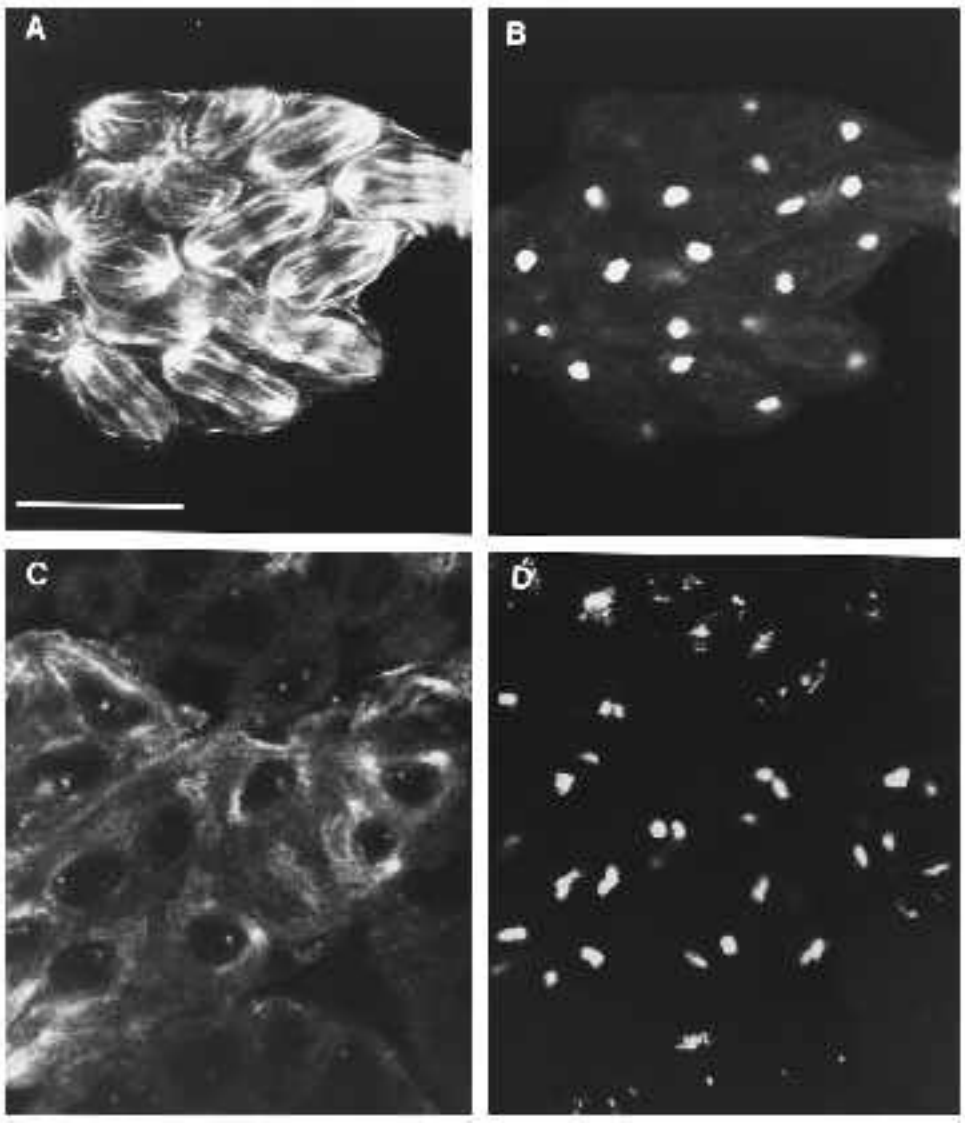

Fig. 4. The meiotic spindle does not form in *twine*^{HB5} testes. The meiotic spindle can be visualised using a monoclonal antibody against tubulin (YL1/2). (A) (probed with YL1/2) and (B) (DNA stained with propidium iodide) show a wild-type cyst at metaphase I. (C) A *twine*^{HB5} cyst in which spindle structures cannot be seen following immunostaining with the YL1/2 monoclonal antibody even though DNA condensation has clearly occurred as shown by the propidium iodide staining shown in (D). Bar, 25 μm.

towards the cortex (Fig. 7A and B); a high power view of one of these thin spindles is shown in the inset to Fig. 7. Such embryos also have free asters of microtubules (see the polar region of the embryo in Fig. 7A). Free asters have been previously described in *Drosophila* embryos in which aberrant mitoses have occured as a result of drug treatment (Raff and Glover, 1988) or mutation (Freeman et al., 1986). The large nuclei that we previously described are also present within these embryos, but are located towards the interior of the embryos. They have no microtubule structures associated with them (Fig. 7C and D).

Unfertilised embryos derived from *twine* mothers undergo some development

After reinitiation and completion of meiosis *Drosophila* oocytes contain the four haploid products of female meiosis, three polar bodies and one pronucleus. After fertilisation the male pronucleus, provided by the sperm, and the female pronucleus fuse to form the zygotic genome. The polar bodies play no further role but remain in the embryo in a decondensed or semicondensed state. The reinitiation of meiosis occurs on entry of the oocyte into the oviduct rather than on fertilisation. Eggs that are not fertilised are thus deposited with the meiotic divisions complete, and development arrests at this point.

We studied unfertilised eggs from *twine*^{HB5} mothers to see if any mitotic abnormalities were present at this stage. We find that approximately one third of the eggs resemble normal unfertilised eggs. In the remainder a variety of phenotypes similar to those described for fertilised embryos were observed. We frequently observed eggs containing thin spindles, similar to the ones described in fertilised embryos, with tiny pieces of chromatin associated with them. Some embryos also contained several large nuclei (data not shown).

DISCUSSION

We have previously described some aspects of the phenotypes resulting from a mutation in the *Drosophila* gene *twine*, one of two homologues of the fission yeast gene

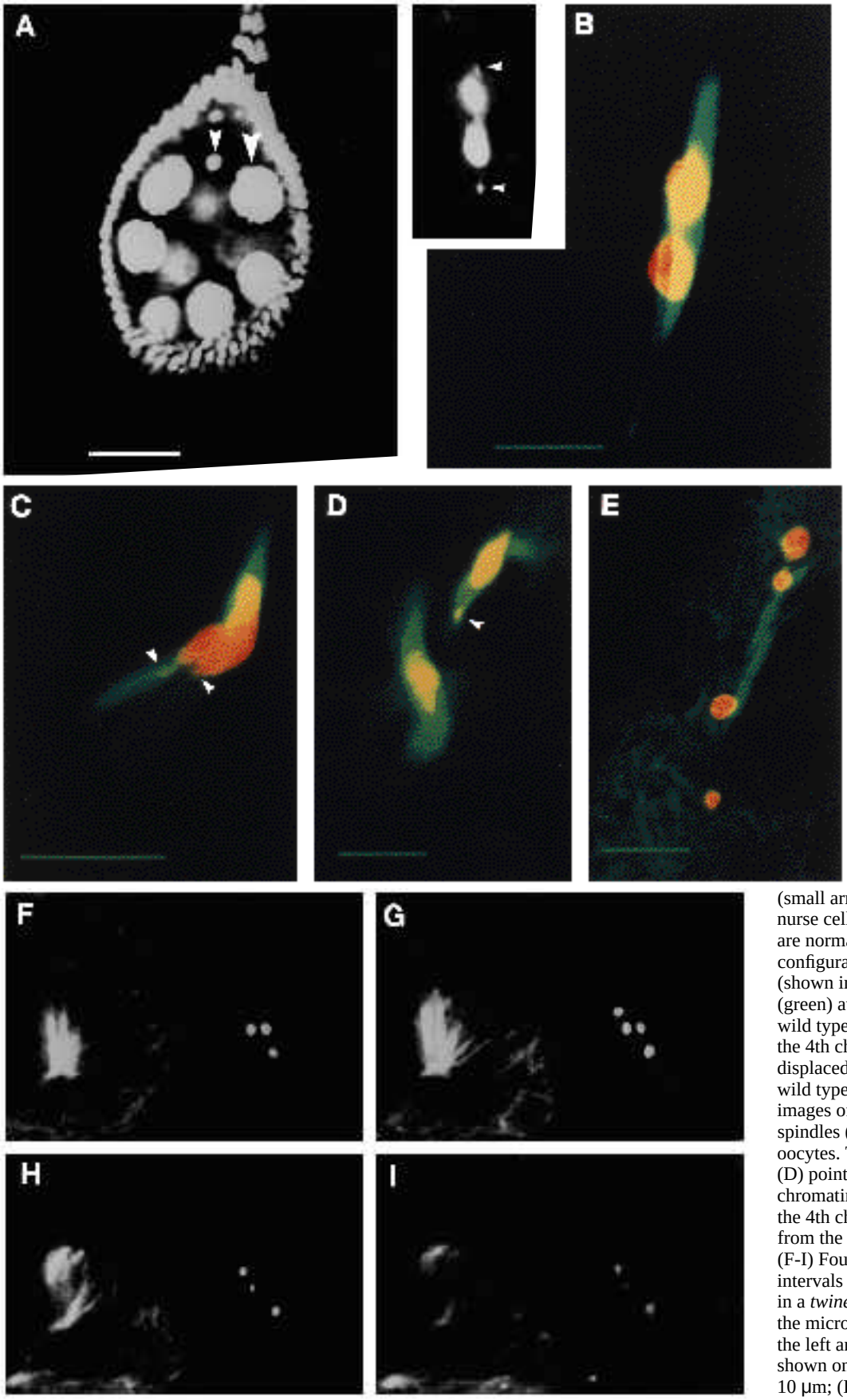

Fig. 5. Meiosis fails to arrest at metaphase I in *twine*^{HB5} oocytes and leads to spindle abnormalities. (A) A stage 7 egg chamber from a *twine*^{HB5} female, the oocyte nucleus (small arrowhead) and the polyploid nurse cell nuclei (large arrowhead) are normal at this stage. (B) The configuration of chromosome (shown in red) and microtubules (green) at the metaphase I arrest in wild type. The inset in (B) shows the 4th chromosomes (arrowheads) displaced towards the poles in the wild type. (C-E) Merged confocal images of chromosomes (red) and spindles (green) from *twine*^{HB5} oocytes. The arrowheads in (C) and (D) point to small pieces of chromatin, which we presume to be the 4th chromosomes 'escaping' from the exchange chromosomes. (F-I) Four sections taken at 1 μm intervals through an abnormal figure in a *twine*^{HB5} oocyte. In each panel the microtubule staining is shown on the left and the DNA staining is shown on the right. Bars: (A and C), 10 μm; (B, D and E), 5 μm.

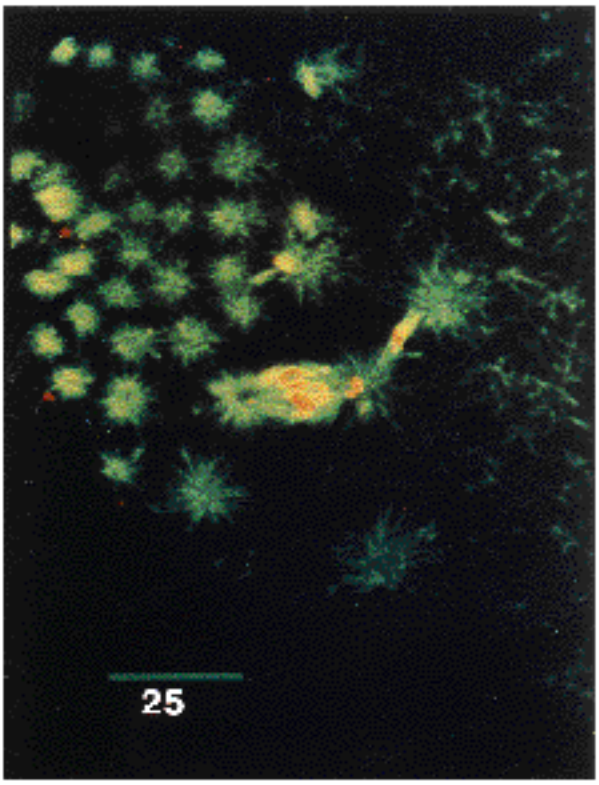

Fig. 6. Normal and thin spindles in embryos derived from *twine*^{HB5} mothers. A merged confocal image showing DNA (red) and tubulin (green) in a region of a *twine*^{HB5}-derived embryo. A normal-sized spindle is seen sharing a pole with a thin spindle. Free asters of microtubules are also visible. Bar, 25 μm.

cdc25 (Jimenez et al., 1990; Alphey et al., 1992). Our previous studies suggested defects in the progression through meiosis in both males and females. In males, the meiotic divisions do not occur and so the cysts of primary spermatocytes remain with 16 cells, although they do undertake considerable further development, including elongation of the nuclei and formation of sperm-like structures. The synthesis of *twine* transcripts in the growing stage of primary spermatocytes suggests a role in regulating the entry into meiosis. This is analogous to the G_2-M transition of the mitotic cycle in which the breakdown of the nuclear envelope, chromosome condensation, and the formation of the spindle are thought to be mediated through p34^{cdc2} kinase, following its activation by dephosphorylation by the *cdc25* phosphatase. The *twine*^{HB5} allele was sequenced and shown to have a missense mutation that results in a conserved proline residue in the tyrosine phosphatase domain of the protein being changed to a leucine residue (Courtot et al., 1992). The mutant phenotype of *twine*^{HB5}/*Df(2L)RN2* is indistiguishable from that in homozygous *twine*^{HB5} flies, indicating that *twine*^{HB5} is an amorphic allele. This was also suggested by the failure of *twine*^{HB5} to rescue a *cdc25*^{ts} mutant of *S. pombe* (Courtot et al., 1991). It therefore seemed likely that p34^{cdc2} kinase regulation was not being correctly activated during meiosis in *twine*^{HB5} mutants. We were therefore surprised to find that, contrary to our expectations and to the report of Courtot et al. (1992), chromosome condensation does occur in mutant *twine*^{HB5} males and moreover it is accompanied by nuclear envelope breakdown. A spindle never forms, however, and so it seems that some aspects of the entry into meiosis can take place whereas others cannot. There are a number of possible explanations for these observations. Perhaps p34^{cdc2} does not mediate all aspects of the G_2-M transition for the entry into male meiosis. This is not without precedent since in *Aspergillus* a kinase encoded by the gene *nimA* (Osmani et al., 1991) appears to mediate some events of the G_2-M transition. Interestingly a gene for a related kinase, *Nek1*, has been identified that is expressed at high levels in meiotic germ cells in mouse (Letwin et al., 1992). Alternatively some other '*cdk*-like' enzyme could mediate some of these steps. Another possibility is that *twine* and *string* independently activate different forms of p34^{cdc2} to mediate entry into meiosis, and thus in the *twine* mutant, the *string*-mediated steps can still occur. However, *string* transcripts are only seen at the apex of the testes in the progenitors of cells that will not undertake meiosis until about 90 hours later. We do not know the distribution of *string* protein in the testes, but its seems unlikely that it would persist for this period of time, since in other systems *cdc25* instability appears to be a key feature of its function as a mitotic regulator. An alternative is that some forms of the p34^{cdc2} complex might not be regulated by the phosphorylation/dephosphorylation of tyrosine 15. In extracts of activated *Xenopus* eggs p34^{cdc2} complexed to cyclin A is not subject to inhibitory phosphorylation of tyrosine 15, in contrast to the p34^{cdc2}/cyclin B complex (Clarke et al., 1992; Devault et al., 1992). If this were the case in the *Drosophila* spermatocyte, *twine* function would only be required to activate the p34^{cdc2}/cyclin B complex. This would then be consistent with growing evidence in support of differing roles for the cyclin A- and cyclin B-associated p34^{cdc2} kinases in modifying microtubule behaviour (Verde et al., 1990; Buendia et al., 1992). The cyclin B-associated enzyme is required to bring about the specific and abrupt shortening of interphase microtubules crucial in the establishment of the spindle, and in several organisms, including *Drosophila*, cyclin B has been demonstrated to associate with the polar regions of the spindle (e.g. see Maldonado-Codina and Glover, 1992). The failure of the spindle to form in *twine* mutants could reflect a specific role in the activation of p34^{cdc2}/cyclin B.

In contrast to the meiotic block seen in *twine*^{HB5} males, meiosis continues abnormally in females. Female meiosis normally arrests at metaphase I in stage 14 of oogenesis and remains blocked until the egg passes through the oviduct. The phenotype that we observe in *twine* mutant females suggests that *twine* function is required to maintain this arrest by keeping p34^{cdc2} dephosphorylated at tyrosine 15 and thereby active. In *Drosophila*, meiotic recombination only occurs in the female. Thus it might be expected that the mechanisms regulating entry into the first meiotic division might differ between the sexes, since recombination requires the assembly of synaptonemal complexes and exchange nodules in what is essentially an extended prophase. The mechanism whereby the meiotic spindle is established in female meiosis is also quite characteristic, and probably explains the differing requirement for *twine* (and p34^{cdc2}) function between male and female meiosis.

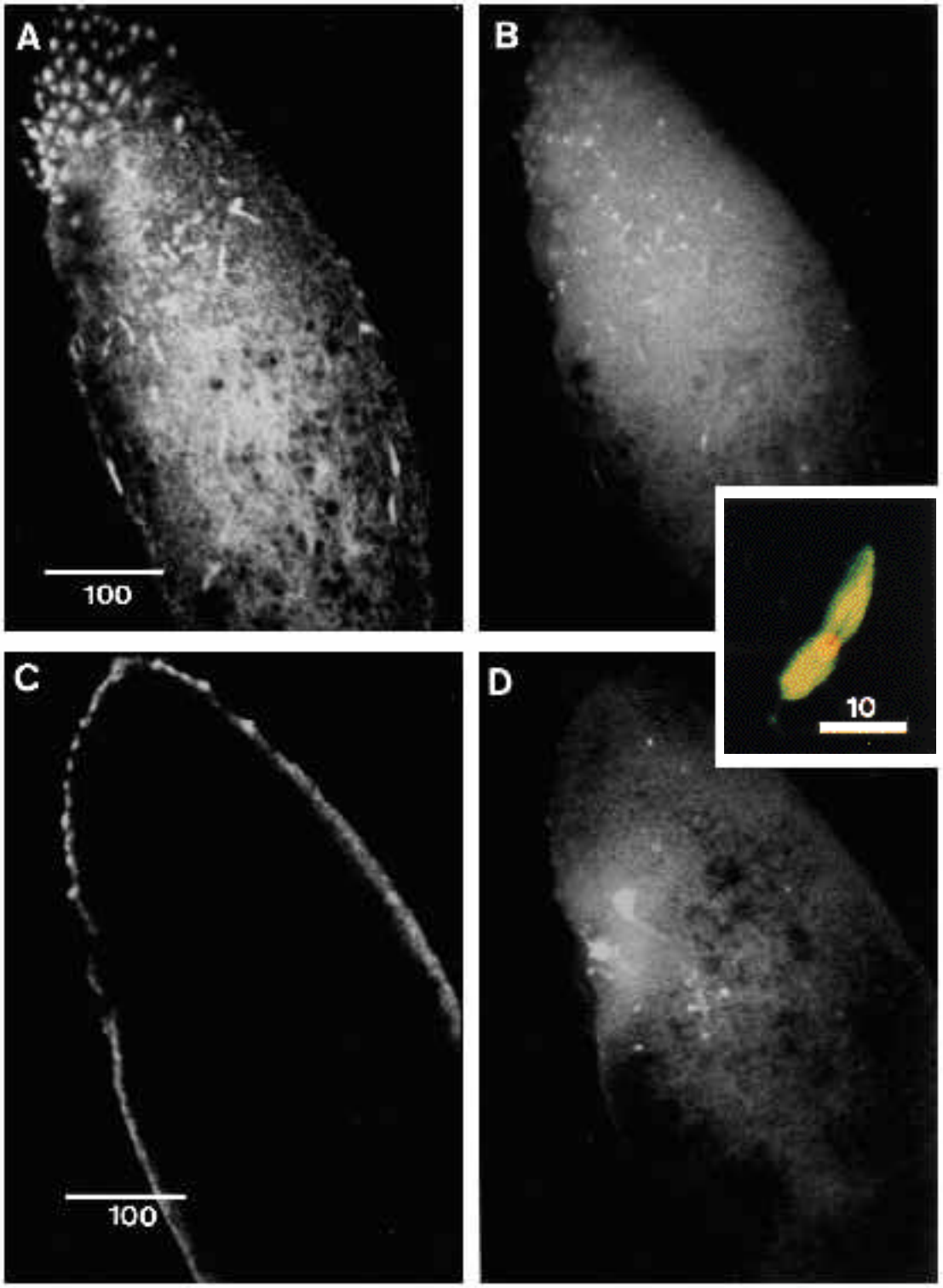

Fig. 7. Thin spindles with associated small pieces of chromatin in *twine*^{HB5} derived embryos. Tubulin (A and C) and DNA (B and D) staining from two focal planes from the same *twine*^{HB5} embryo. Thin spindles are clearly visible at the surface of the embryo (A). Each of these is associated with a small sphere of DNA, probably the 4th chromosomes (B). In the interior of the embryo two large nuclei are visible (D). These are not associated with any microtubular structures (C). The inset shows a higher power view of one of the thin spindles (microtubules stained green) with its associated DNA (red). Bars: (A-D), 100 μm; inset, 10 μm.

A cytological study of spindle assembly in female meiosis led Theurkauf and Hawley (1992) to propose that the major microtubule nucleating activity is provided by paired centromeres of the major chromosomes rather than the centrosomes. Such diverse mechanisms of spindle formation might be expected to be under different regulation, and so spindle formation in the female may not be blocked by the *twine* mutation, as it is in male meiosis. The bundling of microtubules emanating from the chromosomal nucleation points requires the activity of a kinesin-like molecule encoded by the *ncd* gene (Walker et al., 1990; Hatsumi and Endow, 1992; Sequeira et al., 1989). In *ncd* mutants, this bundling is not complete, leading to spindles with broad poles that are often distorted around the metaphase plate, and which resemble the abnormal *twine* spindles. Normally an equilibrium exists at metaphase I in which the chrom-

somes that have undergone recombination remain at the equator still connected through chiasmata that will eventually ensure their correct segregation. The separation of non-exchange chromosomes is controlled in part through the kinesin-like protein encoded by *nod* (Zhang and Hawley, 1990). This imparts a force upon these chromosomes in the direction of the metaphase plate, and is counteracted by a poleward directed force that allows non-exchange chromosomes to move toward the poles in a size-dependent manner. In this way, the tiny fourth chromosomes become positioned between the poles and the equator. This type of arrangement is not seen in the second meiotic metaphase in which all chromosomes align on the metaphase plate before undertaking the equational division. Premature separation of the fourth chromsome is seen in the multiple meiotic-like divisions that occur in *twine* oocytes. If these are repeated attempts at the reductional division, then this would explain the dramatic non-disjunction that occurs during *twine* meiosis. Mutation in *nod* leads to the dissociation of non-exchange chromosomes from the spindle or their premature movement to the pole. Similar events can also be seen in *twine* mutants.

Arrest at metaphase I in female meiosis is normally also dependent upon recombination having taken place to produce chiasmate bivalents (McKim et al., 1993). Thus, in mutants that prevent recombination, the meiotic arrest at metaphase I does not occur. However, the absence of any significant zygotic lethality indicates that meiosis is otherwise normal and relies entirely upon the mechanisms for segregating non-exchange chromosomes. Thus the failure to arrest in *twine* mutants differs profoundly from the effects of mutations preventing meiotic recombination. It has been suggested that the formation of chiasmata leads to the establishment of mechanical tension at the metaphase plate that signals a meiotic block (McKim et al., 1993). The gross abnormalities that we observe in meiosis in *twine* females suggests that its function is likely to be a prerequisite for the block imposed through the mechanism that senses the presence of chiasmata.

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